United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract

Government Performance Results Act Goal: Clean and Safe Water

Significant Research Findings:

Detecting Emerging and CCL-Related Pathogens

Scientific Problem and Policy Issues	This research is designed to provide improved methods for detecting and enumerating emerging and Contaminant Candidate List (CCL)-related microbial pathogens that are potentially transmitted through drinking water. The CCL was developed in response to the 1996 amendments to the Safe Drinking Water Act (SDWA), which required that EPA periodically identify new drinking water contaminants for potential regulation. Emerging pathogens are contaminants that are likely to appear on future CCL lists. The methods developed under this project area are needed to obtain occurrence and exposure data that are required for adequate characterization of potential public health risks. They can also be used to evaluate the effectiveness of various drinking water treatment options or for routine compliance monitoring. The development of policies, guidance and regulations that ensure the availability of safe, pathogen-free drinking water are thus dependent on the data obtained by these improved methods.
Research Approach	The objective for this project area is to develop and evaluate practical, sensitive, and economical technologies for the detection and measurement of specific emerging and CCL-related microbes. Meeting this objective required the development of multiple approaches, including: 1) new cell lines and cell culture methods to identify pathogenic human enteric viruses, protozoa, and bacteria; 2) genomic and immuno-based methods for rapid detection of pathogenic microbes, especially for those organisms which cannot be cultured or which are difficult to culture; 3) scanning and transmission electron microscopy to monitor and identify microbial contaminants and to confirm cell culture and immunoassay results; 4) fluorescence based confocal microscopy techniques to image and identify the ultrastructure of living pathogenic agents; and 5) source water quality indicator systems that accurately predict the presence and source of human fecal contamination in environmental waters.
Results and Implications	This Annual Performance Measure (APM 45) supports FY01 Annual Performance Goal 007 which states: Reduce Uncertainties and

Improve Methods Associated with the Assessment and Control of Risks Posed by Exposure to Microbial Contaminants in Drinking Water with a Focus on the Emerging Pathogens on the CCL."

Significant findings are as follows:

A rapid molecular method has been developed to detect and identify caliciviruses in environmental and drinking water. Recently, this method was used to detect and identify a genogroup 2 calicivirus in well water following a waterborne outbreak. Caliciviruses have been the responsible etiological agent in many waterborne disease outbreaks and probably account for the majority of outbreaks where the etiological agents have not been identified. The Centers for Disease Control estimate that caliciviruses are responsible for greater than 90 percent of all non-bacterial gastroenteritis in the U.S. and worldwide.

A rapid molecular method was developed that will identify all known hepatitis E viral strains in drinking and source waters. The hepatitis E virus is a waterborne emerging pathogen that has caused many outbreaks of illness in the developing world, including Mexico. Thus far, an outbreak of this virus has not been reported in the U.S., however, several sporadic cases have occurred, and there is concern that it will be an emerging infectious disease in this country. An important bearing on this disease is the finding that a variant of the virus is common in hogs in the U.S. It is thought that hogs may serve as a reservoir for human infection, inasmuch as hepatitis E viral isolates from two of the U.S. human cases were more closely related to the U.S. hog hepatitis E virus than to human strains from other parts of the world.

Three new chapters describing virus detection technology have been appended to the U.S. EPA Manual of Methods for Virology. Chapter 14 describes the most widely used viral method for recovering human enteric viruses from water matrices. The method takes advantage of positively charged filters to concentrate viruses from water. Chapter 15 describes a quantal method for assaying culturable waterborne human enteric viruses. The assay differs from the plaque assay in that it is based on a direct microscopic viewing of cells for virus-induced cytopathic effects and has a greater sensitivity. The methods described in Chapters 14 and 15 primarily detect infectious enteroviruses and reoviruses. The enterovirus group includes the coxsackieviruses and echoviruses, two groups that are included on the CCL. Chapter 16 describes procedures for the detection of coliphage in water matrices. Two quantitative procedures and one qualitative presence-absence

procedure are presented. The procedures described have an important consideration as a general indicator of water quality. Currently, hard copies of these chapters are being distributed. In the near future electronic text of these publications will be available in the EPA Microbiology Home page (www.epa.gov/microbes/).

A method has been developed by which a microsporidia species can be identified from processed water samples. The method has been published and is the first report of a flourescent in situ hybridization assay that utilizes a species-specific fluorescent-labeled oligonuclotide probe. Microsporidia are obligate intracellular protozoan parasites that are on the CCL.

Research Collaboration and Publications

Research collaborations are ongoing with several governmental organizations on basic work to develop research tools and to provide organisms for several of the emerging parasites. These collaborators include the World Health Organization, the Centers for Disease Control, the Department of Agriculture (Interagency Agreement # DW12938843), the U.S. Geological Survey (Interagency Agreements # DW14938174 and DW14939295), the University of Cincinnati (Assistance Agreement # CR826758) and the Department of Defense (Interagency Agreement #DW97937961). Other collaborative projects are with Kansas State University which has undertaken the development of a cell culture model for parasitic protozoan propagation, and with the Veteran's Administration Palo Alto Health Care System (Interagency Agreement # DW36938176) and the Johns Hopkins School of Public Health which are developing molecular testing methods for emerging and CCL list parasites. Innovative methods of isolating etiological agents of emerging diseases from large volume water samples are being investigated under a collaborative project with the Marshfield Medical Research and Education Foundation. A examination of calicivirus strains in latrine and well water samples is being conducted in Kenya, Africa in conjunction with the University of Surrey in the United Kingdom (Assistance Agreement # R82860301).

Examples of recent publications from this study include:

Bennett, J.W., Gauci, M.R., LeMoenic, S., Schaefer, III, F.W., and Lindquist, H.D.A. "A comparison of enumeration techniques for *Cryptosporidium parvum* oocysts." J. Parasitol. 85:1165-1168, 1999.

Fout, G.S., Dahling D.R, and Safferman, R.S. "Collecting and processing of water-borne viruses by positive charged filtrationand organic flocculation." In USEPA Manual of Methods for Virology, EPA-600/4-84-013 (N14), U. S. Environmental

Protection Agency, Cincinnati, OH. (April 2001).

Fout, G.S., Dahling D.R, and Safferman, R.S. " Total culturable virus quantal assay." In USEPA Manual of Methods for Virology, EPA-600/4-84-013 (N15), U. S. Environmental Protection Agency, Cincinnati, OH (April 2001).

Grimm, A.C. and Fout, G.S. "Development of a molecular method to identify hepatitis E virus in environmental water." *J. Virol. Meth.* (submitted).

Hester, J.D., Lindquist, H.D.A., Bobst, A.M., and Schaefer III, F.W. "Fluorescent in situ detection of *Encephalitozoon hellem* spores with a 6-carboxyfluorescein-labeled ribosomal RNA-targeted oligonucleotide probe." *J. Eukaryot. Microbiol.* 47:299-308, 2000.

Lindquist H.D.A., Bennett, J.W., Ware, M., Stetler, R.E., Gauci, M., and Schaefer III, F.W. "Testing methods for detection of *Cryptosporidium spp.* in water samples." Supplement to the *Southeast Asian Journal of Tropical Medicine and Public Health* (In press).

Lindquist, H.D.A., Ware, M., Stetler, R.E., Wymer, L., and Schaefer, III, F.W. "A comparison of four fluorescent antibody based methods for purifying, detecting and confirming *Cryptosporidium parvum* in surface waters." *J. Parasitol.* (In press).

Pandian, A, Berg, G., Dahling, D.R., Cashdollar, J.C., Wymer, L., and Fout G. S. and L.Wymer. "Comparison of a new beef extract preparation with beef extract V for eluting and reconcentrating poliovirus 1 adsorbed on 1MDS filters." *J. Virol. Meth.* (Cleared for publication).

Simmons III, O.D., Sobsey, M.D., Heaney, C.D., Schaefer III, F.W., and Francy, D.S. 2001. "Concentration and detection of *Cryptosporidium* oocysts in surface water samples by method 1622 using ultrafiltration and capsule filtration." *Appl. Environ. Microbiol.* 67:1123-1127, 2001.

Schaefer, III, F.W. 2001. "Detection of protozoans and helminths in source and finished drinking water." In *Manual of Environmental Microbiology,* 2nd ed. (Cleared for publication).

Williams, F.P., Stetler, R.E., and Safferman, R.S. "Procedures for detecting coli-phages." In USEPA Manual of Methods for Virology, EPA-600/4-84-013 (N16), U. S. Environmental Protection Agency, Cincinnati, OH (June 2001).

Future Research

Future research will continue to focus on reducing deficiencies in the current technology needed to process and detect emerging and CCL-related pathogens. These studies are of importance to the Office of Water, Agency risk assessors, the scientific community and industry spokes groups as they will enhance the ability to measure the occurrence and exposure of the public to waters contaminated with pathogenic agents and thereby reduce the public health uncertainties.

This research will include the introduction of new technologies, such as combining cell culture and molecular methods in order to identify and characterize the listed pathogenic agents. At the same time, studies will be continuing on the development of faster and simpler indicator methods for evaluating microbial water quality using innovative cultural, immunological, genomic and biochemical approaches. In addition, future research will also be supporting the development of procedures that will differentiate viable from non-viable and infectious from non-infectious microbial pathogens. Other methods will be developed that will aid in identifying the sources of microbial pathogens such as human versus animal contamination.

Contacts for Additional Information

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